

## **AMENDMENTS TO THE CLAIMS**

The following listing of claims will replace all prior versions and listings of claims in the application.

### **LISTING OF CLAIMS**

1. (Currently amended) An oral vaccine comprising at least one of recombinant adhesin protein of *Aeromonas hydrophila* (AHMA), recombinant protein AHMA fragments, and recombinant protein derivatives.

2. (Original) The vaccine of Claim 1 wherein at least one of the recombinant protein fragments and derivatives is emulsified in water-in-oil emulsion.

3. (Original) The vaccine according to Claim 2 wherein said emulsifying oil further comprises organic oil.

4. (Original) The vaccine according to Claim 3 wherein said emulsifying oil further comprises palm oil.

5. (Currently amended) The vaccine according to Claim 2 wherein the proportion of water and oil in the emulsion ~~further comprises~~ is in the ratio of 1:2.

6. (Original) The vaccine according to Claim 2 wherein the proportion of water and oil in the emulsion is equal.

7. (Original) The oral vaccine according to Claim 2 mixed with a binding agent.

8. (Original) The oral vaccine of Claim 7 wherein the binding agent further comprises particulate feed material.

9. (Original) The oral vaccine of Claim 8 wherein the binding agent further comprises high viscosity carboxymethylcellulose.

10. (Original) The oral vaccine of Claim 1 comprising an immunologically effective dose of recombinant AHMA protein.

11. (Currently amended) The oral vaccine according to Claim 1 further comprising recombinant fusion protein from *Ichthyophthirius multifiliis* (FP).

12. (Original) The vaccine of Claim 11 wherein the recombinant proteins are emulsified in a water-in-oil emulsion.

13. (Original) The vaccine according to Claim 12 wherein said emulsifying oil further comprises organic oil.

14. (Original) The vaccine according to Claim 13 wherein said emulsifying oil further comprises palm oil.

15. (Currently Amended) The vaccine according to Claim 12 wherein the proportion of water and oil in the emulsion ~~further comprises~~ is in the ratio of 1:2.

16. (Original) The vaccine according to Claim 12 wherein the proportion of water and oil in the emulsion is equal.

17. (Original) An oral vaccine according to Claim 12 mixed with a binding agent.

18. (Original) The vaccine according to Claim 17 wherein the binding agent further comprises particulate feed.

19. (Original) The vaccine according to Claim 17 wherein the binding agent further comprises carboxymethylcellulose.

20. (Currently amended) The oral vaccine according to Claim 11 comprising immunologically effective dose of at least one of the constituent proteins selected from the group consisting of recombinant protein AHMA, recombinant protein AHMA fragments, and recombinant protein derivatives.

21. (Original) The oral vaccine according to Claim 1 further comprising inactivated viruses elected from a group consisting of guppy reovirus and guppy nervous necrosis virus.

22. (Currently amended) The oral vaccine according to Claim 1 further comprising bacterial antigens or killed bacteria selected from a group consisting of ~~Shiwanella putrefaciens~~ Shewanella putrefaciens, ~~Pseudomonas fluorescens~~ Pseudomonas fluorescens, ~~Vibrio alginolyticus~~ Vibrio alginolyticus and ~~Flexinobacter columnaris~~ Flexibacter columnaris.

23. (Withdrawn) A method of making an oral vaccine comprising the steps of:

a) separately mixing a predetermined amount of at least one of recombinant protein AHMA, recombinant protein AHMA fragments, and recombinant protein derivatives and whole recombinant protein AHMA, either singly or in combination with at least one antigen selected from the group consisting of recombinant protein FP, guppy reovirus, guppy nervous necrosis virus, *Shiwanella putrefaciens*, *Pseudomonas fluorescens*, *Vibrio alginolyticus* and

*Flexinobactor columnaris* in a predetermined volume of at least one of water and saline.

b) vigorously mixing a pre-determined volume of organic oil with (a) to form an emulsion.

c) optionally, adding a binding agent to emulsion (b) with gentle stirring to obtain the consistency of a paste, and

d) optionally, adding particulate feed to (c) to obtain a particulate oral vaccine.

24. (Withdrawn) The method according to Claim 23 wherein the organic oil further comprises palm oil.

25. (Withdrawn) The method according to Claim 23 wherein the binding agent further comprises particulate feed.

26. (Withdrawn) The method according to Claim 23 wherein the binding agent further comprises high viscosity carboxymethylcellulose.

27. (Currently amended) The vaccine prepared by the method according to Claim 23 ~~48~~ wherein the computed dosage of recombinant AHMA in the vaccine ranges between 7  $\mu\text{g/g}$  and 150  $\mu\text{g/g}$  body weight of the recipient.

28. (Currently amended) The oral vaccine prepared by the method according to Claim 23 48 wherein the amount of recombinant AHMA is between 15 $\mu$ g/g and 20  $\mu$ g/g body weight of the recipient.

29. (Currently amended) The oral vaccine prepared by the method according to Claim 23 48 wherein the amount of recombinant AHMA is 17  $\mu$ g/g body weight of the recipient.

30. (Currently amended) The vaccine prepared by the method according to Claim 23 48 wherein the computed dosage of recombinant FP in the vaccine ranges between 7  $\mu$ g/g and 150 $\mu$ g/g body weight of the recipient.

31. (Currently amended) The oral vaccine prepared by the method according to Claim 23 48 wherein the amount of recombinant FP is between 15 $\mu$ g/g and 20  $\mu$ g/g body weight of the recipient.

32. (Currently amended) The oral vaccine prepared by the method according to Claim 23 48 wherein the amount of recombinant FP is 17  $\mu$ g/g body weight of the recipient.

33. (Currently amended) The vaccine prepared by the method according to Claim ~~23~~ 48 wherein the computed dosage of at least one of viral proteins and inactivated virus in the vaccine ranges between  $10^3$  and  $10^6$  viral particles/g body weight of the recipient.

34. (Currently amended) The oral vaccine prepared by the method according to Claim ~~23~~ 48 wherein the amount of at least one of viral protein and inactivated virus is  $10^5$  viral particles/g body weight of the recipient.

35. (Currently amended) The vaccine prepared by the method according to Claim ~~23~~ 48 wherein the computed dosage of at least one of inactivated bacterial and an equivalent amount of bacterial antigens in the vaccine ranges between  $10^5$  cfu/g and  $10^7$  cfu/g body weight of the recipient.

36. (Currently amended) The oral vaccine prepared by the method according to Claim ~~23~~ 48 wherein the amount of at least one of inactivated bacteria and an equivalent amount of bacterial antigens in the vaccine is  $2.5 \times 10^6$  cfu/g body weight of the recipient.

37. (Withdrawn) A method of treating a species in need of such treatment against aquatic pathogens comprising administering an immunologically effective dose of the vaccine according to Claim 23.

38. (Withdrawn) A method according to Claim 37, wherein said animal is an aquatic species.

39. (Withdrawn) A method according to Claim 38, wherein the aquatic species is fish.

40. (Withdrawn) A method according to Claim 39, wherein the fish is a guppy.

41. (Withdrawn) A method according to Claim 39, wherein the fish is a blue gourami.

42. (Withdrawn) A method according to Claim 39, wherein the fish is a goldfish.

43. (Withdrawn) A fish immunized with the oral vaccine of Claim 23.

44. (Withdrawn) An edible product comprising fish immunized or treated with the vaccine according to Claim 25.

45. (Original) The oral vaccine according to Claim 11 further comprising inactivated viruses elected from a group consisting of guppy reovirus and guppy nervous necrosis virus.



46. (Currently amended) The oral vaccine according to Claim 11 further comprising bacterial antigens or killed bacteria selected from a group consisting of ~~Shewanella putrefaciens~~ Shewanella putrefaciens, ~~Pseudomonas fluorescens~~ Pseudomonas fluorescens, ~~Vibrio alginolyticus~~ Vibrio alginolyticus and ~~Flexibacter columnaris~~ Flexibacter columnaris.

47. (Currently amended) The oral vaccine according to Claim 21 further comprising bacterial antigens or killed bacteria selected from a group consisting of ~~Shewanella putrefaciens~~ Shewanella putrefaciens, ~~Pseudomonas fluorescens~~ Pseudomonas fluorescens, ~~Vibrio alginolyticus~~ Vibrio alginolyticus and ~~Flexibacter columnaris~~ Flexibacter columnaris.

48. (New) An oral vaccine prepared by a method comprising the steps of:

a) separately mixing a predetermined amount of at least one of recombinant adhesin protein of *Aeromonas hydrophila* (AHMA), recombinant protein AHMA fragments, and recombinant protein derivatives and whole recombinant protein AHMA, either singly or in combination with at least one antigen selected from the group consisting of recombinant fusion protein from *Ichthyophthirius multifiliis* (FP), guppy reovirus, guppy nervous necrosis virus, *Shewanella putrefaciens*, *Pseudomonas fluorescens*, *Vibrio alginolyticus* and

*Flexibacter columnaris* in a predetermined volume of at least one of water and saline,

b) vigorously mixing a pre-determined volume of organic oil with (a) to form an emulsion,

c) optionally, adding a binding agent to emulsion (b) with gentle stirring to obtain the consistency of a paste, and

d) optionally, adding particulate feed to (c) to obtain a particulate oral vaccine.